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Porphyromonas bronchialis sp. nov. Isolated from Intraoperative Bronchial Fluids of a Patient with Non-Small Cell Lung Cancer

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Porphyromonas strains, including *Porphyromonas*-like strains, have been isolated from oral and various other systemic infections. The characterization of such strains is a crucial issue, because such information contributes to both the taxonomy of anaerobic bacteria and the clinical aspects of infectious diseases. We previously isolated four *Porphyromonas*-like strains from intraoperative bronchial fluids of a patient with non-small cell lung cancer. This study aimed to characterize the genetic, biochemical and chemotaxonomic aspects of these isolates. Each strain only grew under anaerobic conditions and their colony morphology was convex, 0.1-1.0 mm in diameter, light gray, and slightly glistening colony, with no black or brown pigmentation on blood agar plates after five-day incubation. The pigmentation was helpful to differentiate the isolates from other *Porphyromonas*, as most of *Porphyromonas* species show the pigmentation. In the 16S rRNA gene phylogenetic analysis (98% sequence identity of isolates indicates the same species), the four isolates were closely related to one another (99.7-100.0%), but not related to *Porphyromonas* (*P.*) *catoniae*, the closest species (96.9%). In addition, the DNA-DNA hybridization data revealed less than 16% similarity values between a representative isolate and the *P. catoniae*, indicating that the strains were genetically independent. Biochemically, the isolates could be differentiated from closely related species, i.e., *P. catoniae*, *P. gingivalis*, *P. gulae*, and *P. pogonae*, with trypsin activity (negative only in the isolates) and leucine arylamidase activity (positive only in the isolates). We therefore propose a new species to include these isolates: *Porphyromonas bronchialis* sp. nov.

Keywords: bronchial fluids; DNA-DNA hybridization; human clinical specimen; *Porphyromonas*; 16S rRNA sequence
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Introduction

Numerous bacterial strains of Gram-negative, non-spore-forming, rod-shaped, obligate anaerobic bacteria, belonging to the genus *Porphyromonas*, have been isolated from oral infections and many other infections throughout the body, e.g., abscesses in the buttock and groin areas, soft tissue infections, amniotic fluid, umbilical cord, pelvic abscesses, vulvovaginitis, and other infected tissues (Shah and Collins 1988; Krieg 2011; Kawamura et al. 2015). Within the genus *Porphyromonas*, 16 species are currently recognized, i.e., *Porphyromonas asaccharolyticus*, *Porphyromonas bennonis*, *Porphyromonas cangingivalis*, *Porphyromonas canoris*, *Porphyromonas catoniae*, *Porphyromonas circumdentaria*, *Porphyromonas crevioricanis*, *Porphyromonas endodontalis*, *Porphyromonas gingi-*

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valis, *Porphyromonas gingivicanis*, *Porphyromonas gulae*, *Porphyromonas levii*, *Porphyromonas macacae*, *Porphyromonas pogonae*, *Porphyromonas somerae* and *Porphyromonas uenosis*.

Taxonomy within the genus *Porphyromonas* has progressed in these days; however, many strains remain uncharacterized (Fournier et al. 2001; Bemis et al. 2011). The characterization of such not-yet-identified strains, e.g., how different from established *Porphyromonas* species on the genetical, biochemical, and chemotaxonomical basis, is essential for the completion of *Porphyromonas* taxonomy, and thus attracting attentions of researchers. Furthermore, information on such not-yet-identified strains has been, in general, thought to contribute greatly to the clinical aspects of infectious diseases.

Porphyromonas-like strains have been isolated from oral cavities, urogenital system, and intestinal tracts of their hosts (Willems and Collins 1995; Fournier et al. 2001; Finegold et al. 2004; Hardham et al. 2005; Summanen et al. 2005, 2009; Mikkelsen et al. 2008; Bemis et al. 2011; Filioussis et al. 2015; Kawamura et al. 2015). Usually, most of the uncharacterized strains are known to be unavailable between different laboratories. Recently, as one of the means of obtaining uncharacterized *Porphyromonas*-like strains, we quantified and identified bacteria in intraoperative bronchial fluids of nine elderly patients using a bronchoscopic micro-sampling probe and a well maintained anaerobic glove box system, and successfully isolated 79 predominant facultative and obligate anaerobes (Hasegawa et al. 2014). Among the 79 strains, there were four *Porphyromonas*-like organisms; however, to date, their phenotypic and genetic characteristics remain to be fully defined. Thus, the purpose of the present study was to characterize the genetic, biochemical, and chemotaxonomic aspects of the four strains. In this paper, we report the results of the phenotypic analysis, 16S rRNA gene phylogenetic analysis, DNA-DNA hybridization, G + C content and cellular fatty acid analysis, in order to determine the taxonomic position of the four strains.

Materials and Methods

Bacterial strains and growth conditions

Four isolates of a new candidate species were used in the present study; these four *Porphyromonas* isolates, PAGU 1600^T to 1603 (PAGU, School of Pharmacy, Aichi Gakuin University), were also numbered, Tohoku 18-2 to 18-5, respectively (Table 1). They were isolated from intraoperative bronchial fluids of a patient (male, aged 74 years) with non-small cell lung cancer, i.e., adenocarcinoma in the left upper lobe and squamous cell carcinoma in the left lower lobe, at Tohoku University Hospital, Sendai, Japan, utilizing a bronchoscopic micro-sampling probe and an anaerobic glove box system (model AZ-Hard, containing 80% N_2, 10% H_2, and 10% CO_2; Hirasawa, Tokyo, Japan). Informed consent was obtained from the patient, and this study was approved by the Research Ethics Committee of Tohoku University Graduate School of Dentistry, Sendai, Japan, as described previously (Hasegawa et al. 2014). The following type strains were also used in this study; *Porphyromonas gingivalis* PAGU 1218^T (= JCM, Japan Collection of Microorganisms 12257^T), *Porphyromonas asaccharolyticus* PAGU 1229 ^{T} (= JCM 6326^T), *Porphyromonas catoniae* PAGU 1627^T (= JCM 13863^T), and *Porphyromonas gulae* PAGU 1794^T (= JCM 13865^T). All strains were grown on Brucella Blood Agar with Hemin and Vitamin K (Difco) plates, at 37°C under an anaerobic atmosphere consisting of 80% N_2, 10% CO_2 and 10% H_2, generated using an Anoxomat Mark II anaerobic system (Mart Microbiology, Drachten, Netherlands). Black or brown pigmentation on the agar plates after 5 to 14 days incubation at 37°C was checked by the visual inspection.

Phenotypic analysis

The biochemical traits of each of these isolates were characterized using API-ZYM and Rapid ID32A systems (Sysmex bioMérieux Co., Ltd., Tokyo, Japan), according to the manufacturer's instructions. Catalase activity was monitored by adding a drop of 3% hydrogen peroxide to a microbial smear. Fermentation tests for each sugar were performed using Fastidious Anaerobe Broth (FAB; Lab M Ltd., Lancashire, UK) supplemented with 1% glucose, lactose, or maltose. After 3 days of incubation at 37°C under anaerobic conditions, pH of the medium was measured. A pH value lower than 5.5 was considered to indicate a positive fermentation result, pH of 5.5-6.0 was considered to be weakly positive for fermentation, and a pH value higher than 6.0 indicated no fermentation (Holdeman et al. 1977). Volatile fatty acids (C_2-C_6), acetoin, diacetyl alcohols (C_2-C_5), and free acids or methyl derivatives of lactic, succinic, phenyl acetic, phenyl propionic, and formic acids, produced in peptone-yeast extract-glucose broth (PYG), were assayed by gas chromatography, as described previously (Hoshino et al. 1978; Hoshino and Sato 1986).

Table 1. Strains used in the present study.

Species name	Strain number	Isolated from
Porphyromonas bronchialis	PAGU 1600^T (= Tohoku 18-2)	Intraoperative bronchial fluids
Porphyromonas bronchialis	PAGU 1601 (= Tohoku 18-3)	Intraoperative bronchial fluids
Porphyromonas bronchialis	PAGU 1602 (= Tohoku 18-4)	Intraoperative bronchial fluids
Porphyromonas bronchialis	PAGU 1603 (= Tohoku 18-5)	Intraoperative bronchial fluids
Porphyromonas gingivalis	PAGU 1218^T (= JCM 12257^T)	Human gingival sulcus
Porphyromonas assacharolyticus	PAGU 1229^T (= JCM 6326^T)	Empyema
Porphyromonas catoniae	PAGU 1627^T (= JCM 13863^T)	Human gingival crevice of adult patient
Porphyromonas gulae	PAGU 1794^T (= JCM 13865^T)	Wolf gingival sulcus

16S rRNA gene phylogenetic analysis

Phylogenetic analysis was carried out using 16S rRNA gene sequences, as described previously (Kawamura et al. 2009). Briefly, the 16S rRNA gene (> 1,300 bases) sequences for each isolate were determined using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Tokyo, Japan) and the 3130 Genetic Analyzer (Applied Biosystems). Other sequences used for alignment and calculating the identity levels were obtained from the DNA Data Bank of Japan (DDBJ) database (http://www.ddbj.nig.ac.jp/). Multiple sequence alignments of DNA sequences were carried out using Clustal X software (http://www.clustal.org/). Phylogenetic distances were estimated using the neighbor-joining method, and the phylogenetic tree was drawn using TreeView (http://www.ddbj.nig.ac.jp/search/help/tv-setup.html) and NJPlot (http://www.softpedia.com/get/Science-CAD/NJPlot.shtml) software.

DNA-DNA hybridization and determination of G + C content

Quantitative microplate DNA-DNA hybridization was carried out, as described previously (Kawamura et al. 1999). Hybridization experiments were carried out at 34°C (optimal conditions) or 44°C (stringent conditions) in 2× SSC and 50% formamide. The mol% G + C contents of the DNA were determined by high-performance liquid chromatography as described previously (Kawamura et al. 1998).

Cellular fatty acid analysis

Cellular fatty acids of each isolate were liberated with 10% KOH/methanol at 100°C for 30 min, followed by extraction with n-hexane. After methylation with 10% trimethylsilyldiazomethane in n-hexane (Nacalai Tesque Inc., Kyoto, Japan), methyl ester derivatives of fatty acids were analyzed by gas chromatography-mass spectrometry (GC/MS) (Li et al. 2003; Tomida et al. 2011; Kawamura et al. 2012).

Scanning electron microscopy

Bacterial cell shape was observed by scanning electron microscopy, as described previously (Kawamura et al. 2015). Briefly, bacterial colonies were suspended in phosphate-buffered saline, washed three times, and smeared onto a cover glass. Bacteria were fixed with 0.1 M sodium cacodylate containing 2.5% (v/v) glutaraldehyde for 1 h at 4°C. After washing with 0.1 M sodium cacodylate, samples were dehydrated in a graded series of ethanol solutions ranging from 50% (v/v) ethanol in distilled water to 100% ethanol. All samples were coated with 5 nm of platinum by magnetron sputtering and examined by scanning electron microscopy (JXA-8530FA; JOEL, Tokyo, Japan).

Results and Discussion

The genetic, biochemical, and chemotaxonomic aspects of the four unidentified strictly anaerobic strains, isolated from intraoperative bronchial fluids of a patient with pulmonary carcinoma, were characterized in this study.

Genetic analysis

The phylogenetic analysis of 16S rRNA sequences demonstrated that the four isolates belong within the genus *Porphyromonas*. It is generally accepted that a 98% 16S rRNA sequence identity is an indication that isolates are members of the same species (Stackebrandt and Goebel 1994). In this study, these four isolates were closely related to one another (99.7-100.0% sequence identity), but not related to *Porphyromonas* (*P.*) *catoniae* PAGU 1600^T, the closest species (96.9% sequence identity, Fig. 1). In addition, in the whole-genome DNA-DNA hybridization studies, a threshold of 70% or greater similarity indicates a relationship at the species level (Wayne et al. 1987). In our study, the DNA-DNA hybridization data revealed less than 16% DNA-DNA similarity values between a representative isolate PAGU 1600^T and the *P. catoniae* type strain (Table 2), indicating that the strains were genetically independent. Our hybridization data appear to be reliable, as the similarity values between *P. gingivalis* and *P. gulae* observed in our experiments (42.2-55.6%, Table 2) were in almost the same range as those (53-65%) reported by Kato et al. (1997). Based on these data, we concluded that the *Porphyromonas* isolates from intraoperative bronchial fluids fell into a single taxon, but did not belong to any established species of the genus.

The G + C mol% of a representative isolate PAGU 1600^T was 56.50 ± 0.67 mol%, which were clearly distinct from other non-pigmented *Porphyromonas* species, i.e., *P. catoniae* (49 mol%; Willems and Collins 1995), *P. bennonis* (58 mol%; Summanen et al. 2009), and *P. pogonae* (43.00 ± 0.62 mol%; Kawamura et al. 2015).

Cellular fatty acid and fermentation end product analyses

Fatty acid analysis revealed that 3OH-iso-C17:0 (18.8-20.4%) predominated, followed by anteiso-C15:0, C16:0, and C18:0 in almost equal amounts (11.1-12.2%, 9.5-10.6%, and 9.6-11.2%, respectively), which could be separated from other non-pigmented *Porphyromonas* species, i.e., *P. catoniae* (C15:0 iso, C15:0 antiiso; Willems and Collins 1995; Kawamura et al. 2015), *P. bennonis* (C15:0 DMA, 3OH-C14:0; Summanen et al. 2009; Kawamura et al. 2015), and *P. pogonae* (C15:0 iso, C15:0 anteiso, 3OH-C17:0 iso; Kawamura et al. 2015) (Table 3). Fermentation end product data showed that propionic acid was the most abundant product (3.94-13.10 mM), followed by acetic acid (1.84-5.26 mM), which differed from other non-pigmented *Porphyromonas* species, i.e., *P. catoniae* (propionic and succinic acids, followed by acetic, iso-valeric and lactic acids; Willems and Collins 1995; Kawamura et al. 2015), *P. bennonis* (acetic and succinic acids; Summanen et al. 2009; Kawamura et al. 2015), and *P. pogonae* (succinic, propionic and acetic acids, followed by iso-valeric and lactic acids; Kawamura et al. 2015) (Table 3).

Colony and cell morphology, and phenotypic analysis

All four isolates showed very similar colony morphology; convex, 0.1-1.0 mm in diameter, light gray, slightly glistening colony, and did not show black or brown pigmentation (Table 3) on Brucella Blood Agar with Hemin and Vitamin K (Difco) plate after five days incubation under anaerobic condition at 37°C, which were distinct

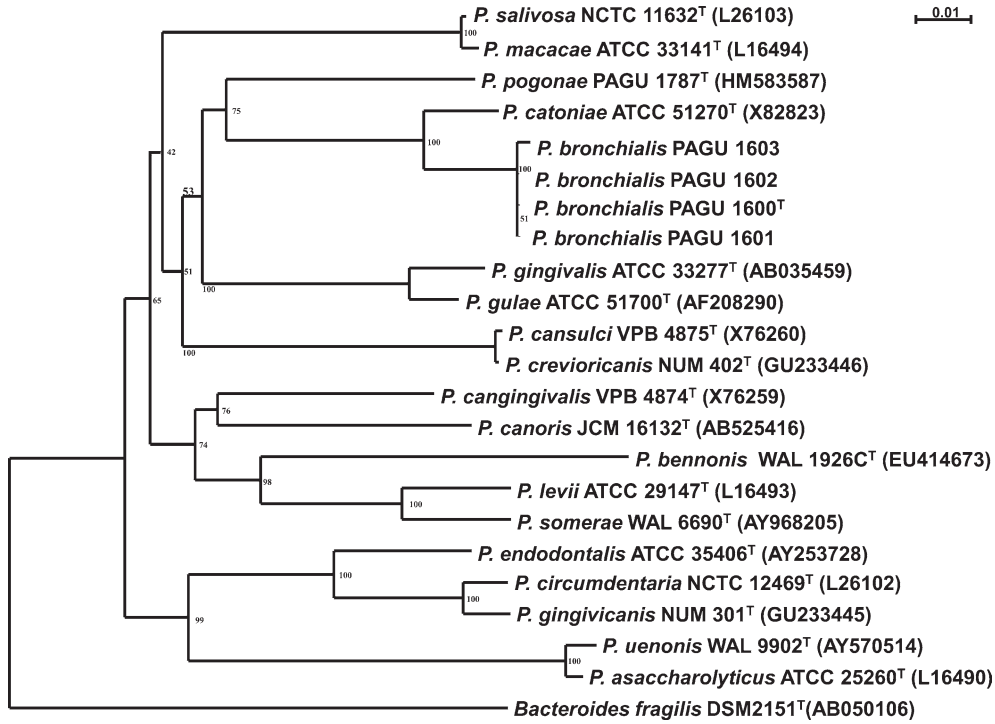

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of *Porphyromonas bronchialis* within the genus *Porphyromonas*. The numbers at the branching points are bootstrap percentages (based on 1,000 replications). *Bacteroides fragilis* DSM 2151^T is used as an out group. Scale bar represents one inferred nucleotide substitution per 100 nucleotides. GenBank/DDBJ accession numbers are given in parenthesis, and type strains are indicated as ^{T} (superscript notations). In addition to PAGU and JCM, the following acronyms of Culture Collections are used in Fig. 1.
NCTC; National Collection of Type Cultures, Central Public Laboratory Service, London, UK.
ATCC; American Type Culture Collection, Manassas, VA.
VPB; Veterinary Pathology and Bacteriology Collection, University of Sydney, New South Wales, Australia.
NUM; Nihon University School of Dentistry at Matsudo, Chiba, Japan.
WAL; Wadsworth Anaerobe Laboratory, Wadsworth Hospital Center, Los Angeles, CA.
DSM; DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Table 2. Whol-genome DNA-DNA hybridization assay between *P. bronchialis* and closely related species of the genus *Porphyromonas*.

	P. bronchialis PAGU 1600^T		*P. gingivalis* PAGU 1218^T		*P. catoniae* PAGU 1627^T		*P. gulae* PAGU1794^T	
	Optimal	Stringent	Optimal	Stringent	Optimal	Stringent	Optimal	Stringent
P. bronchialis PAGU 1600^T	100.0	100.0	3.9 ± 0.6	2.1 ± 0.1	14.6 ± 0.4	8.5 ± 0.7	4.7 ± 0.5	3.0 ± 0.2
P. gingivalis PAGU 1218^T	3.3 ± 0.6	1.4 ± 0.2	100.0	100.0	3.1 ± 0.4	1.6 ± 0.3	55.6 ± 1.3	50.6 ± 2.9
P. catoniae PAGU 1627^T	15.3 ± 2.6	10.0 ± 2.0	3.2 ± 0.6	1.9 ± 0.1	100.0	100.0	4.7 ± 0.6	2.7 ± 0.1
P. gulae PAGU1794^T	1.8 ± 0.1	1.6 ± 0.4	49.1 ± 4.7	42.2 ± 3.4	1.9 ± 0.2	0.5 ± 0.0	100.0	100.0

DNA-DNA hybridization was carried out using the photobiotin microplate method. Values are percentage results from hybridization at 34°C (optimal conditions) and 44°C (stringent conditions). Data represent means ± standard deviation of three experiments.

from closely related species of pigmented *Porphyromonas*, such as *P. gingivalis* (Krieg 2011) and *P. gulae* (Fournier et al. 2001). In addition, no pigment production was observed in the four isolates even after incubation for more than 10 to 14 days, which differed from non-pigmented *Porphyromonas* species, *P. bennonis* (Summanen et al. 2009; Kawamura et al. 2015) and *P. pogonae* (Kawamura et al. 2015). Colonies did not show red fluorescence under UV (366 nm) light, similar to *P. catoniae*, *P. gingivalis*, and *P. gulae*, but in contrast to *P. pogonae* (Table 3). Catalase tests were negative for all isolates, similar to *P. catoniae* and *P. gingivalis*, but in contrast to *P. gulae* and *P. pogonae* (Table 3). Cells from each isolate appeared microscopically as small, approximately 0.5-0.8 μm × 0.8-2.5 μm, Gram-negative rods, and coccobacilli.

The isolates could be differentiated from closely related species, such as *P. catoniae*, *P. gingivalis*, *P. gulae*, and *P. pogonae* by measuring trypsin activity (negative only in the isolates, but positive in the other four species) and leucine arylamidase activity (positive only in the isolates,

Table 3. Differential characteristics of *P. bronchialis* sp. nov. and closely related species of the genus *Porphyromonas.*

	P. bronchialis	*P. catoniae*[a]	*P. gingivalis*[a]	*P. gulae*[a]	*P. pogonae*[a]
Pigment production	−	−	+	+	−[b]
Fluorescence	−	−	−	−	+
Indole	−	−	+	+	+
Catalase	−	−	−	w	+
Enzyme activity (commercial tests)[c]					
α-Fucosidase	+	+	−	−	−
α-Galactosidase	−	d	−	−	−
β-Galactosidase	+	+	−	+	+
N-Acetyl-*β*-glucosaminidase	+	+	+	+	+
Chymotrypsin	w	d	−	w	+
Trypsin	−	d	+	+	+
Pyroglutamic acid arylamidase	−	−	−	−	+
Glycine arylamidase	−	−	−	−	+
Leucine arylamidase	+	−	−	−	−
Fermentation of:					
Glucose[d]	−	+	−	−	+
Lactose[d]	−	+	−	−	+
Maltose[d]	−	+	−	−	w
Mannose[e]	d (1/2)	+	−	−	d (6/7)
Raffinose[e]	+	+	−	−	d (5/7)
Metabolic end products from PYG[f]	A, P, iv, s	a, P, iv, l, S	A, P, ib, B, IV, s, pa	B, IV, S, PA	S, P, A, iv, l
Major long-chain fatty acids	3OH-iso-C17:0, anteiso-C15:0, C18:0, C16:0	C15:0 iso, C15:0 antiiso	C15:0 iso	na[g]	C15:0 iso, C15:0 anteiso, 3OH-C17:0 iso

[a]Data of *P. catoniae*, *P. gingivalis*, *P. gulae* and *P. pogonae* were taken from Krieg (2011), Fournier et al. (2001), Kawamura et al. (2015) and our own results.

+: more than 90% of the strains are positive, d: 89-11% of the strains are positive (and their actual numbers of positive/tested strains were described in parentheses), −: less than 10% of the strains are positive, w: weakly positive reaction.

[b]Negative but weakly positive after incubation for more than 10 to 14 days.

[c]Reaction in the API ZYM system and/or Rapid ID32A system (bioMérieux).

[d]Reaction in the Fastidious Anaerobe Broth (Lab M Ltd.).

[e]Reaction in the Rapid ID32A system (bioMérieux).

[f]A, acetic acid; P, propionic acid; IB, isobutyric acid; B, butyric acid; IV, isovaleric acid; L, lactic acid; S, succinic acid; PA, phenylacetic acid. Capital letters represent an amount of metabolic product equal to or greater than 10 mM from peptone-yeast extract-glucose (PYG); small letters represent an amount of metabolic product less than 10 mM.

[g]Data are not available for the present.

but negative in the other four species) (Table 3). In addition to the pigmentation and catalase activity (described above), *α*-fucosidase activity, and fermentation tests for glucose, lactose, maltose, mannose, and raffinose as substrates were also helpful to differentiate the isolates from other members of the genus *Porphyromonas* (Table 3).

Clinical implication and summary

It has been suggested that silent aspiration of bacteria occurs in elderly patients with pulmonary carcinoma and that the risk of pneumonia in these patients is high (Hasegawa et al. 2014); however, neither postoperative pneumonia nor carcinoma recurrence was observed in any patients in that study, whose intraoperative bronchial fluids were the source of the isolates. Thus, the pathological characteristics of this species of *Porphyromonas* (such as on pulmonary carcinoma as well as aspiration pneumonia) are unknown, and further study is necessary.

All four isolates collected from human bronchial fluids are novel members of the genus *Porphyromonas*, and here, we propose the name *Porphyromonas bronchialis* sp. nov. The summary of the taxonomic properties of the species is described as follows.

Description of *Porphyromonas bronchialis* sp. nov.

Porphyromonas bronchialis (L. pl. n. bronchia, the bronchial tubes; L. fem. suff.-alis, suffix used with the sense of pertaining to; N.L. fem. adj. bronchialis, pertaining to the bronchi, coming from the bronchi [bronchial fluids]).

The description below is based on our own observations.

Cells are Gram-negative, non-spore-forming rods, 0.5-

0.8 μm × 0.8-2.5 μm in size. They grow under anaerobic conditions only.

Convex, 0.1-1.0 mm in diameter, light gray, and slightly glistening colonies appeared on Brucella Blood Agar with Hemin and Vitamin K (Difco) plates after 5 days of incubation under anaerobic conditions at 37°C. The differential characteristics of this organism and related species are described as follows (and Table 3). Black or brown pigmentation was not observed. No fluorescence was observed under long-wave UV light. The organism is catalase-, indole-, urease-, and nitrate-negative. Positive reactions were exhibited for α-fucosidase, β-galactosidase, N-acetyl-β-glucosaminidase, leucine arylamidase, and chymotrypsin (weak positive) and fermentation tests for raffinose and mannose (1/2 strains). The organism is negative for other tests that were run on the API-ZYM and Rapid-ID32A systems.

The metabolic end products identified by gas chromatography were propionic acid followed by acetic acid. The major fatty acids detected in the strains were 3OH-iso-C17:0, anteiso-C15:0, C16:0, and C18:0. The G + C mol% content was 56.50 ± 0.67%.

We propose that this type strain be designated PAGU 1600^{T}. The organism was isolated from human bronchial fluids.

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Conflict of Interest

The authors declare no conflict of interest.

References

Bemis, D.A., Greenacre, C.B., Bryant, M.J., Jones, R.D. & Kania, S.A. (2011) Isolation of a variant *Porphyromonas* sp. from polymicrobial infections in central bearded dragons (*Pogona vitticeps*). *J. Vet. Diagn. Invest*., **23**, 99-104.

Filioussis, G., Petridou, E., Karavanis, E. & Frey, J. (2015) Pyogranulomatous pneumonia in goats caused by an undescribed *Porphyromonas* species, "*Porphyromonas katsikii*". *J. Clin. Microbiol*., **53**, 795-798.

Finegold, S.M., Vaisanen, M.L., Rautio, M., Eerola, E., Summanen, P., Molitoris, D., Song, Y., Liu, C. & Jousimies-Somer, H. (2004) *Porphyromonas uenonis* sp. nov., a pathogen for humans distinct from *P. asaccharolytica* and *P. endodontalis*. *J. Clin. Microbiol*., **42**, 5298-5301.

Fournier, D., Mouton, C., Lapierre, P., Kato, T., Okuda, K. & Ménard, C. (2001) *Porphyromonas gulae* sp. nov., an anaerobic, gram-negative coccobacillus from the gingival sulcus of various animal hosts. *Int. J. Syst. Evol. Microbiol*., **51**, 1179-1189.

Hardham, J., Dreier, K., Wong, J., Sfintescu, C. & Evans, R.T. (2005) Pigmented-anaerobic bacteria associated with canine periodontitis. *Vet. Microbiol*., **106**, 119-128.

Hasegawa, A., Sato, T., Hoshikawa, Y., Ishida, N., Tanda, N., Kawamura, Y., Kondo, T. & Takahashi, N. (2014) Detection and identification of oral anaerobes in intraoperative bronchial fluids of patients with pulmonary carcinoma. *Microbiol. Immunol*., **58**, 375-381.

Holdeman, L.V., Cato, E.P. & Moore, W.E.C. (1977) Procedures and specific tests; biochemical tests. In *Anaerobe Laboratory Manual*, 4th ed., Virginia Polytechnic Institute and State University, Blacksburg, VA, pp. 125-128.

Hoshino, E., Frölander, F. & Carlsson, J. (1978) Oxygen and the metabolism of *Peptostreptococcus anaerobius* VPI4330-1. *J. Gen. Microbiol*., **107**, 235-248.

Hoshino, E. & Sato, M. (1986) Production and degradation of formate by *Veillonella dispar* ATCC 17745. *J. Dent. Res*., **65**, 903-905.

Kato, T., Hirai, K., Okuda, D., Fournier, D., Menard, C. & Mouton, C. (1997) Animals are not reservoir for the human periodontal pathogen Porphyromonas gingivalis. *Dent. Jpn.*, **33**, 27-30.

Kawamura, Y., Fujiwara, N., Naka, T., Mitani, A., Kubota, H., Tomida, J., Morita, Y. & Hitomi, J. (2012) Genus *Enhydrobacter* Staley et al. 1987 should be recognized as a member of the family *Rhodospirillaceae* within the class *Alphaproteobacteria*. *Microbiol. Immunol*., **56**, 21-26.

Kawamura, Y., Hou, X.G., Todome, Y., Sultana, F., Hirose, K., Shu, S.E., Ezaki, T. & Ohkuni, H. (1998) *Streptococcus peroris* sp. nov. and *Streptococcus infantis* sp. nov., new members of the *Streptococcus mitis* group, isolated from human clinical specimens. *Int. J. Syst. Bacteriol*., **48**, 921-927.

Kawamura, Y., Kuwabara, S., Kania, S.A., Kato, H., Hamagishi, M., Fujiwara, N., Sato, T., Tomida, J., Tanaka, K. & Bemis, D.A. (2015) *Porphyromonas pogonae* sp. nov., an anaerobic but low concentration oxygen adapted coccobacillus isolated from lizards (*Pogona vitticeps*) or human clinical specimens, and emended description of the genus *Porphyromonas* Shah and Collins 1988. *Syst. Appl. Microbiol.*, **38**, 104-109.

Kawamura, Y., Tomida, J., Morita, Y., Naka, T., Mizuno, S. & Fujiwara, N. (2009) '*Lysobacter enzymogenes* ssp. *cookii*' Christensen 1978 should be recognized as an independent species, *Lysobacter cookii* sp. nov. *FEMS Microbiol. Lett*., **298**, 118-123.

Kawamura, Y., Whiley, R.A., Shu, S.E., Ezaki, T. & Hardie, J.M. (1999) Genetic approaches to the identification of the mitis group within the genus *Streptococcus*. *Microbiology*, **145**, 2605-2613.

Krieg, N.R. (2011) Family *Porphyromonadaceae*. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, 2nd ed., edited by Krieg, N.R., Staley, J.T., Brown, D.R., Hedlund, B.P., Paster, B.J., Ward, N.L., Ludwig, W. & Whitman, W.B. Springer, New York, NY, pp. 61-85.

Li, Y., Kawamura, Y., Fujiwara, N., Naka, T., Liu, H., Huang, X., Kobayashi, K. & Ezaki, T. (2003) *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. *Syst. Appl. Microbiol*., **26**, 523-528.

Mikkelsen, D., Milinovich, G.J., Burrell, P.C., Huynh, S.C., Pettett, L.M., Blackall, L.L., Trott, D.J. & Bird, P.S. (2008) Phylogenetic analysis of *Porphyromonas* species isolated from the oral cavity of Australian marsupials. *Environ. Microbiol*., **10**, 2425-2432.

Shah, H.N. & Collins, M.D. (1988) Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus, *Porphyromonas*. *Int. J. Syst. Bacteriol*., **38**, 128-131.

Stackebrandt, E. & Goebel, B.M. (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol*., **44**, 846-849.

Summanen, P.H., Durmaz, B., Väisänen, M.L., Liu, C., Molitoris, D., Eerola, E., Helander, I.M. & Finegold, S.M. (2005)

Porphyromonas somerae sp. nov., a pathogen isolated from humans and distinct from *Porphyromonas levii*. *J. Clin. Microbiol*., **43**, 4455-4459.

Summanen, P.H., Lawson, P.A. & Finegold, S.M. (2009) *Porphyromonas bennonis* sp. nov., isolated from human clinical specimens. *Int. J. Syst. Evol. Microbiol.*, **59**, 1727-1732.

Tomida, J., Sakamoto, D., Sugita, T., Fujiwara, N., Naka, T., Hamada, M., Morita, Y. & Kawamura, Y. (2011) *Branchiibius cervicis* sp. nov., a novel species isolated from patients with atopic dermatitis. *Syst. Appl. Microbiol*., **34**, 503-507.

Willems, A. & Collins, M.D. (1995) Reclassification of *Oribaculum catoniae* (Moore and Moore 1994) as *Porphyromonas catoniae* comb. nov. and emendation of the genus *Porphyromonas*. *Int. J. Syst. Bacteriol*., **45**, 578-581.

Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackbrandt, E., Starr, M.P. & Trüper, H.G. (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol*., **37**, 463-464.